## Homework 02

In this homework we work on images, colormaps and sensor calibration.

```
begin
using ColorSchemes # for different colors maps
using Plots # for heatmap
using TestImages # for testimage
using ImageShow # for better rendering of inline images in Pluto
using Colors # for Gray
using PlutoUI
end
```

#### my\_show

```
my_show(arr::AbstractArray{<:Real}; set_one=false, set_zero=false)</pre>
```

Displays a real valued array. Brightness encodes magnitude. Works within Jupyter and Pluto.

#### **Keyword args**

- set\_one=false divides by the maximum to set maximum to 1
- set\_zero=false subtracts the minimum to set minimum to 1

```
my_show(arr::AbstractArray{<:Real}; set_one=false, set_zero=false)

Displays a real valued array . Brightness encodes magnitude.

Works within Jupyter and Pluto.

## Keyword args

* 'set_one=false' divides by the maximum to set maximum to 1

* 'set_zero=false' subtracts the minimum to set minimum to 1

"""

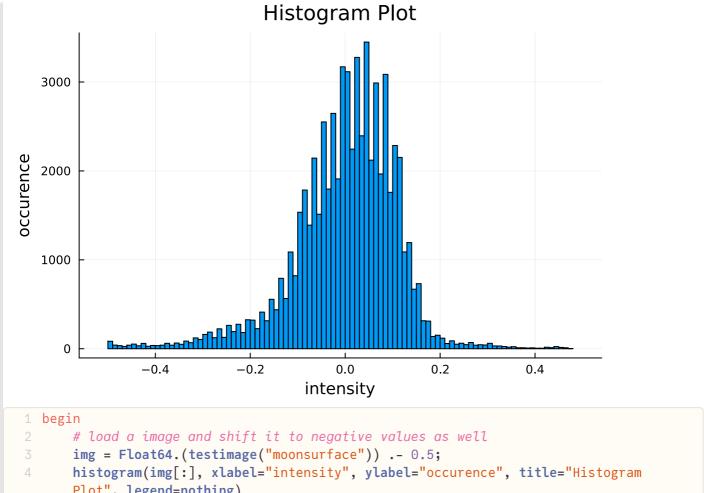
function my_show(arr::AbstractArray{<:Real}; set_one=true, set_zero=false)

arr = set_zero ? arr .- minimum(arr) : arr

arr = set_one ? arr ./ maximum(arr) : arr

Gray.(arr)

end</pre>
```



```
Plot", legend=nothing)
5 end
```

# 1. Colormaps

Very often in microscopy, we look at grayscale images. We already learnt that we can use Gray. (img) for that. However, that is only a very simple viewer. In general, there are much more sophisticated ones like View5D.jl or Napari.jl.

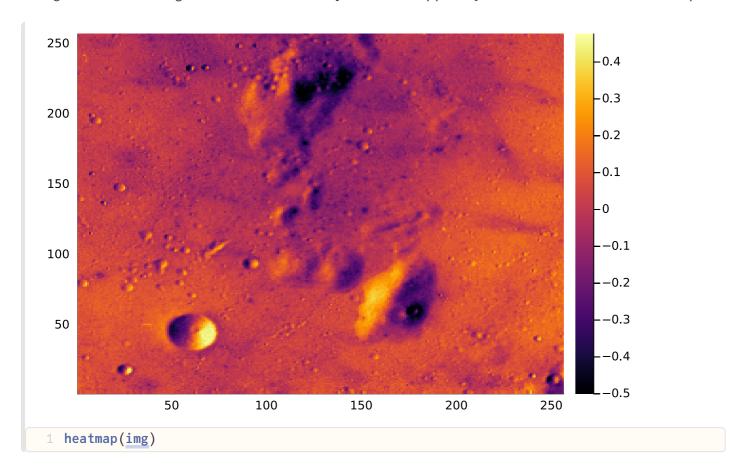
As you can see below, Gray fails pretty quickly and also does not make use of our human color vision.



Gray.(img)

## 1.1 Heatmap

img contains also negative values which Gray does not support by default. A nicer tool is heatmap.



# 1.2 Task - Equal Intensity Color Map

We now want to create a RGB color map which always has the same intensity.

Hence, green\_value + red\_value + blue\_value = const for all different inputs.

#### to\_equal\_intensity\_tuple

```
to_equal_intensity_tuple(value::T) where T
```

Returns for a given scalar input a tuple of equal intensity. There is no unique solution, we only require that the sum is always 1. For example, below would be a valid solution.

#### **Examples**

```
julia> to_equal_intensity_tuple(1.0)
(0.04465819873852073, 0.3333333333333333333336, 0.6220084679281465)

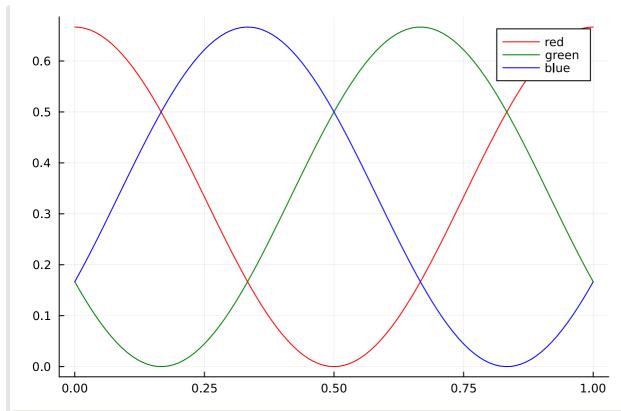
julia> to_equal_intensity_tuple(0.0)
(0.044658198738520505, 0.33333333333333333333, 0.6220084679281462)

julia> to_equal_intensity_tuple(0.3f0)
(0.26402956f0, 0.6503522f0, 0.08561832f0)
```

```
0.00
       to_equal_intensity_tuple(value::T) where T
4 Returns for a given scalar input a tuple of equal intensity.
5 There is no unique solution, we only require that the sum is always 1.
6 For example, below would be a valid solution.
8 ## Examples
9 '''julia-repl
10 julia> to_equal_intensity_tuple(1.0)
11 (0.04465819873852073, 0.3333333333333326, 0.6220084679281465)
13 julia> to_equal_intensity_tuple(0.0)
14 (0.044658198738520505, 0.333333333333333, 0.6220084679281462)
16 julia> to_equal_intensity_tuple(0.3f0)
  (0.26402956f0, 0.6503522f0, 0.08561832f0)
   111
19 """
20 function to_equal_intensity_tuple(x::T) where T
       # with the function signature we can access T (which is the type of 'value')
       # 0 has type Int
       # 1.0 has type Float64
       # 1.0f0 has type Float32
       if x < 0 \mid | x > 1
           error("value must be within [0,1]")
       end
       # fix those three functions such that the property is achieved
       # maybe think about periodic functions ;)
       red_map(x) = (T(1) .+ cos(T(2\pi) .* x)) / T(3)
       green_map(x) = (T(1) \cdot + \cos(T(2\pi) \cdot * x + T(2\pi/3))) / T(3)
       blue_map(x) = (T(1) .+ cos(T(2\pi) .* x + T(4\pi/3))) / T(3)
       return (red_map(x), green_map(x), blue_map(x)) # TODO, return the correct tuple!
38 end
```

```
1 typeof(Float32.(2\pi/3))
```

### Maybe a visualization helps



```
begin
unzip(a) = map(x->getfield.(a, x), fieldnames(eltype(a)))
x = 0:0.01:1
r,g,b = unzip(to_equal_intensity_tuple.(x))
plot(x, r, color=:red, label="red")
plot!(x, g, color=:green, label="green")
plot!(x, b, color=:blue, label="blue")
end
```

## 1.2 Test

# intensity is 1

```
1 using PlutoTest

② all(to_equal_intensity_tuple(0.0f0) .≈ to_equal_intensity_tuple(1.0f0))

1 # cyclic
2 PlutoTest.@test all(to_equal_intensity_tuple(0f0) .≈ to_equal_intensity_tuple(1f0))

③ to_equal_intensity_tuple(0.1) != to_equal_intensity_tuple(0.2)

1 PlutoTest.@test to_equal_intensity_tuple(0.1) != to_equal_intensity_tuple(0.2)

① 1 ≈ sum(to_equal_intensity_tuple(rand(0.0f0:1.0f0)))
```

PlutoTest.@test 1 ≈ sum(to\_equal\_intensity\_tuple(rand(0f0:1f0)))

```
  all(1 .≈ sum.(to_equal_intensity_tuple.(rand(0.0:1.0, 100))))

1 # test for many values
2 PlutoTest.@test all(1 .≈ sum.(to_equal_intensity_tuple.(rand(0.0:1.0, 100))))
```

### Check if the output is always 0<=output<=1

Side Note: it took quite a while to engineer this line :D

```
bbb

PlutoTest.@test bbb
```

### **Type Stability**

4 end

The test might be wrong. Check what happens with expresions like  $2\pi$  / 3. Which type do they have? How can we convert them to different types?

```
NTuple{3, Float32} == typeof(to_equal_intensity_tuple(rand(Float32.(0.0:1.0))))

1 PlutoTest.@test NTuple{3, Float32} == typeof(to_equal_intensity_tuple(rand(Float32.
(0.0:1.0))))
```

```
NTuple{3, Float64} == typeof(to_equal_intensity_tuple(rand(Float64.(0.0:1.0))))

PlutoTest.@test NTuple{3, Float64} == typeof(to_equal_intensity_tuple(rand(Float64.(0.0:1.0)))))
```

## 1.3 Register Color Map

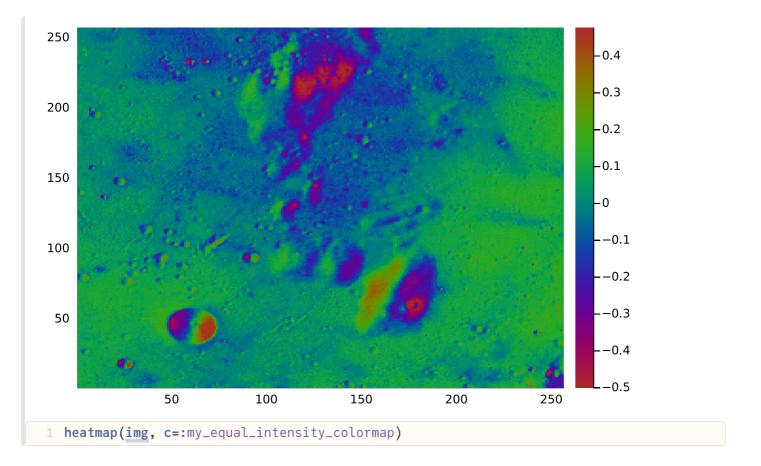
We are able to create valid outputs, but we still need to pass that to the colormap mechanism. We need to register the colormap so that we can load it with heatmap.

You don't need to know the details, but if you'd like understand, you find those in ColorSchemes.jl.

```
create_equal_intensity_colormap (generic function with 1 method)

1 function create_equal_intensity_colormap()
2    values = range(0, 1, length=128)
3    return map(x -> RGB(x...), to_equal_intensity_tuple.(values))
```

```
begin
my_equal_intensity_colormap = create_equal_intensity_colormap()
loadcolorscheme(:my_equal_intensity_colormap, my_equal_intensity_colormap)
end
```



## 1.4 Task

Now we want to create a colormap, which maps values between 0 and 0.5 to blue values and all values above 0.5 to red. Don't let you confuse by negativ and positive intensities. loadcolorschemes needs the values within the interval [0, 1] and will automatically scale it to the image.

#### to\_negative\_positive\_tuple

```
to_negativ_positive_tuple(value::T) where T
```

### **Examples**

```
julia> to_negative_positive_tuple(0.0)
(0.0, 0.0, 1.0)

julia> to_negative_positive_tuple(0.25)
(0.5, 0.5, 1.0)

julia> to_negative_positive_tuple(0.5)
(1.0, 1.0, 1.0)

julia> to_negative_positive_tuple(0.75)
(1.0, 0.5, 0.5)

julia> to_negative_positive_tuple(1.0)
(1.0, 0.0, 0.0)
```

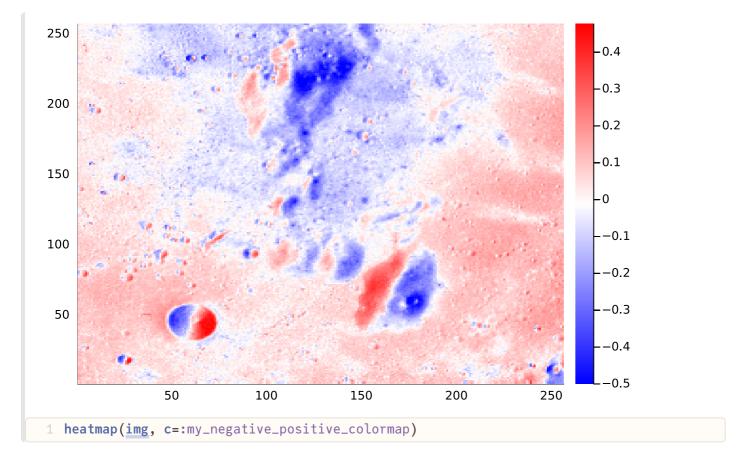
```
\Pi\Pi\Pi
       to_negativ_positive_tuple(value::T) where T
5 ## Examples
6 111
7 julia> to_negative_positive_tuple(0.0)
8 (0.0, 0.0, 1.0)
10 julia> to_negative_positive_tuple(0.25)
11 (0.5, 0.5, 1.0)
13 julia> to_negative_positive_tuple(0.5)
14 (1.0, 1.0, 1.0)
16 julia> to_negative_positive_tuple(0.75)
17 (1.0, 0.5, 0.5)
19 julia> to_negative_positive_tuple(1.0)
  (1.0, 0.0, 0.0)
22 """
23 function to_negative_positive_tuple(x::T) where T
       if x < 0 | | x > 1
           error("value must be within [0,1]")
       end
       if x > 0.5
           x_r = T(1)
           x_g = T(2) * (T(1) - x)
           x_b = T(2) * (T(1) - x)
           return (x_r, x_g, x_b)
       end
       if x <= 0.5
           x_r = T(2) * x
```

Try to show again a heatmap image. The procedure is exactly the same above, except that you need to exchange the core part of the color generation.

```
create_negative_positive_colormap (generic function with 1 method)
```

```
function create_negative_positive_colormap()
values = range(0, 1, length=128)
return map(x -> RGB(x...), to_negative_positive_tuple.(values))
end
```

```
begin
my_negative_positive_colormap = create_negative_positive_colormap()
loadcolorscheme(:my_negative_positive_colormap, my_negative_positive_colormap)
end
```



### **1.4 Test**

```
to_negative_positive_tuple(0.0) == (0.0, 0.0, 1)

1 PlutoTest.@test to_negative_positive_tuple(0.0) == (0.0, 0.0, 1)
```

```
to_negative_positive_tuple(0.5) == (1.0, 1.0, 1.0)

1 PlutoTest.@test to_negative_positive_tuple(0.5) == (1.0, 1.0, 1.0)

• to_negative_positive_tuple(0.75) == (1.0, 0.5, 0.5)

1 PlutoTest.@test to_negative_positive_tuple(0.75) == (1.0, 0.5, 0.5)

• to_negative_positive_tuple(0.25) == (0.5, 0.5, 1)

1 PlutoTest.@test to_negative_positive_tuple(0.25) == (0.5, 0.5, 1)

• typeof(to_negative_positive_tuple(0.25f0)) == Tuple{Float32, Float32, Float32}

1 PlutoTest.@test typeof(to_negative_positive_tuple(0.25f0)) == Tuple{Float64, Float64}

• typeof(to_negative_positive_tuple(0.0)) == Tuple{Float64, Float64, Float64, Float64, Float64, Float64}

1 PlutoTest.@test typeof(to_negative_positive_tuple(0.0)) == Tuple{Float64, Float64, Float64, Float64, Float64, Float64, Float64}
```

### 2 Sensor Simulation

to\_negative\_positive\_tuple(1.0) == (1.0, 0.0, 0.0)

PlutoTest.@test to\_negative\_positive\_tuple(1.0) == (1.0, 0.0, 0.0)

In this part, we want to simulate a sensor. Later, we try to calibrate our artifical sensor.

In short: Each pixel in the sensor measures a number of photons ( $n_photons$ ) which is affected by Poisson noise. Afterwards, the measured value is altered by additive Gauss noise with a certain standard deviation  $\sigma$ . This value is then converted to ADU units (analog to digital units) with a certain gain (linear conversion factor). Additionally, each sensor has an offset which is finally added.

```
begin
using PoissonRandom # for poisson noise ('pois_rand')
using Statistics # for mean, var
end
```

### 2.1 Task

Fill in the missing parts.

#### simulate\_pixel

```
simulate_pixel(n_photons; read_σ=5, offset=0.5, gain=0.1)
```

In this function we simulate a single pixel. It transforms a photon number to a digital unit (ADU).

The input is a integer number of photons which is then altered with poisson noise (use pois\_rand for that). This number is changed with additive Gauss noise (see HWO1). Finally, the result of this operation is multiplied by the linear gain. At the end, we add offset.

- n\_photons: number of photons
- read\_o: additive read noise of the sensor (applied before gain)
- gain: how much digital output per photon
- offset: how much digital offset

### Example Outputs - can vary in your case!

```
julia> simulate_pixel(10)
0.8570431305838304

julia> simulate_pixel(10)
2.060753446522642

julia> simulate_pixel(10)
1.020461460534897

julia> simulate_pixel(100)
8.96276245560446

julia> simulate_pixel.(ones((3,3)))
3×3 Matrix{Float64}:
0.396078 0.96849 -0.0387851
1.09982 0.210089 0.685605
0.606973 1.42573 -0.675297
```

```
simulate_pixel(n_photons; read_σ=5, offset=0.5, gain=0.1)

In this function we simulate a single pixel.

It transforms a photon number to a digital unit (ADU).

The input is a integer number of photons which is then altered with poisson noise (use 'pois_rand' for that).

This number is changed with additive Gauss noise (see HW01).

Finally, the result of this operation is multiplied by the linear 'gain'.

At the end, we add 'offset'.

* 'n_photons': number of photons
* 'read_σ': additive read noise of the sensor (applied before 'gain')

* 'gain': how much digital output per photon

* 'offset': how much digital offset
```

```
20 ## Example Outputs - can vary in your case!
21 '\'julia
22 julia> simulate_pixel(10)
23 0.8570431305838304
25 julia> simulate_pixel(10)
26 2.060753446522642
28 julia> simulate_pixel(10)
29 1.020461460534897
31 julia> simulate_pixel(100)
32 8.96276245560446
34 julia> simulate_pixel.(ones((3,3)))
35 3×3 Matrix{Float64}:
36 0.396078 0.96849
                         -0.0387851
    1.09982
              0.210089
                        0.685605
    0.606973 1.42573
                         -0.675297
   * * *
   0.00
41 function simulate_pixel(n_photons; read_σ=5, offset=0.5, gain=0.1)
       poisson_noise = pois_rand.(n_photons)
        gaussian_noise = read_o .* randn(size(n_photons))
       noise = poisson_noise .+ gaussian_noise
        gained = noise .* gain
       signal = gained .+ offset
       return signal
single_output = 11.36955308442801
 1 single_output = simulate_pixel(100)
123.33030000000014
   mean(simulate\_pixel.(ones((1000,)) * 123, read\_\sigma=0, offset=0.0123, gain=1))
▶ [110.7, 135.3]
 1 [123 - 12.3, 123 + 12.3]
116.06178078078086
 1 var(simulate_pixel.(ones((1000,)) * 123, read_\sigma=0, offset=0.0123, gain=1))
▶ [110.7, 135.3]
 1 [123 - 12.3, 123 + 12.3]
12.364908822646608
 1 std(simulate_pixel.(zeros((1000,)) * 123, read_\sigma=12.3, offset=0, gain=1))
▶ [11.07, 13.53]
   [12.3 - 1.23, 12.3 + 1.23]
535.0860767446889
   std(simulate_pixel.(zeros((1000,)) * 123, read_\sigma=12.3, offset=0, gain=42))
```

```
▶ [464.94, 568.26]

1 [516.6 - 51.66, 516.6+51.66]
```

### **2.1 Test**

```
• 0.0123 \approx simulate_pixel(0, read_\sigma = 0, offset = 0.0123, gain = 1)
 1 # check offset
 2 PlutoTest.@test 0.0123 ≈ simulate_pixel(0, read_σ=0, offset=0.0123, gain=1)
lacktriangle pprox (123, mean(((x
ightarrowsimulate_pixel.(x; read_\sigma = 0, offset = 0.0123, gain = 1))).(ones((100,)) *
1 # check mean poisson
 2 PlutoTest.@test 123 \approx mean((x -> simulate_pixel.(x; read_\sigma=0, offset=0.0123,
   gain=1)).(ones((100,)) * 123)) rtol=0.1
lacktriangle pprox (123, var(((x\rightarrowsimulate_pixel.(x; read_\sigma = 0, offset = 0.0123, gain = 1))).(ones((100,)) * 1
1 # check variance poisson
 2 PlutoTest.@test 123 \approx var((x -> simulate_pixel.(x; read_\sigma=0, offset=0.0123, gain=1)).
    (ones((100,)) * 123)) rtol=0.1
lacktriangle pprox (12.3, std(((x
ightarrowsimulate_pixel.(x; read_\sigma = 0, offset = 0.0123, gain = 1))).(ones((100,)) *
 1 # check readnoise
 2 PlutoTest.@test 12.3 \approx std((x -> simulate_pixel.(x; read_\sigma=0, offset=0.0123,
   gain=1)).(ones((100,)) * 123)) rtol=0.1
lacktriangle pprox (42 * 12.3, std(((x
ightarrowsimulate_pixel.(x; read_\sigma = 12.3, offset = 0, gain = 42))).(zeros((1000)
 1 # check gain
 2 PlutoTest.@test 42 * 12.3 ≈ std((x -> simulate_pixel.(x; read_σ=12.3, offset=0,
   gain=42)).(zeros((1000,))*123)) rtol=0.1
```

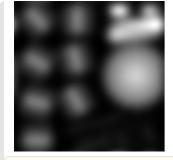
## 2.2 Variance Mean Projection

Now we want to do a Variance Mean Projection to fit the values of offset and gain since we often don't know them for real sensors.

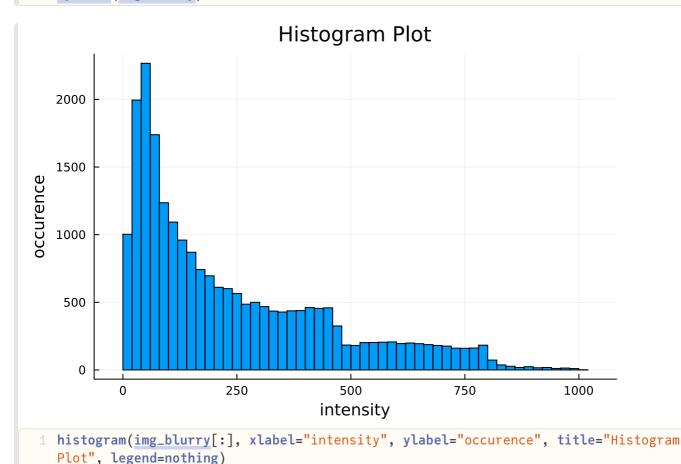
For this test, you take a sample and in practice you defocus your microscope heavily. Via that trick, you have an image which covers all values between very dark and very bright. Hence, we expect that our image sensors both measures a large dynamic range.

```
1 using FourierTools
```

#### That's an ideal image, where we have both very bright and very dark regions



```
1 # our blurry sample which emits 1000 photons in maximum
2 my_show(img_blurry)
```



## 2.2 Task - Apply to image

Now apply the function simulate\_pixel to img\_blurry. Use the automatic broadcasting of Julia for that.

```
simulated_img =
150×150 Matrix{Float64}:
 2.99083
         3.50343
                  1.89752 2.89792 ...
                                        7.65918
                                                  5.49777
                                                            3.98531
                                                                     2.11414
                                                  4.96713
                                                            4.085
                                                                      2.98867
         3.12868
                  2.63481
                           4.21995
                                        7.20419
 5.13804
         3.40469 4.12338
                                                            4.97596 4.86995
                           4.32181
                                        8.94944
                                                  7.24168
 3.04647
                  2.37724
                                       12.492
                                                  8.80551
                                                            7.68381
 4.45162
         5.29623
                           3.67055
                                                                     6.81927
                  3.50683
                                        9.82668
                                                            8.11079
 5.87267
         4.12868
                           4.81572
                                                 10.982
                                                                     7.0015
                  5.29048 3.90158
                                       15.2217
                                                 11.3741
                                                           10.9411
 5.52869 4.98652
                                                                      7.27603
 5.79101 6.42081
                  5.58361 4.27117
                                       16.9599
                                                 13.8018
                                                            9.90212
                                                                     9.64191
                                        2.67628
         4.24614
                  5.71966
                           6.39614
                                                  1.80562
                                                             2.25036
 4.3552
                                                                      2.61056
                  4.29555
                                                             2.79016
 3.97601 4.28687
                                                  4.17655
                                                                      3.22355
                           6.99228
                                        2.83758
                  3.70017
                                                             2.79034
 4.0145
         3.8391
                           5.22811
                                        3.26172
                                                   2.60752
                                                                      3.53797
         4.22781 4.25234
                                        1.98373
 2.82407
                           6.72661
                                                   2.68762
                                                             2.99539
                                                                      3.23128
 1.49067
          2.81211
                  2.60929
                           3.58231
                                        3.56308
                                                   3.75088
                                                             3.04687
                                                                      2.70705
 3.23915
         2.62102 4.48338
                           4.12253
                                        6.02629
                                                   5.0067
                                                             5.11273
                                                                     3.697
 1 simulated_img = simulate_pixel.(img_blurry) # TODO, fix that line such that it uses
    'simulate_pixel'. Use broadcasting mechanism
```

In practice we would need to take a few images (like 10)

Hence we repeat the image 10 times digitally

The output is an array with size(imgs) == (150, 150, 10)

Apply the same trick (broadcasting) for a 2D image to this 3D image

```
begin
imgs = repeat(img_blurry, outer=(1, 1, 10));
images = simulate_pixel.(imgs; offset, read_σ, gain);
end;

begin
continuous imgs = repeat(img_blurry, outer=(1, 1, 10));
images = simulate_pixel.(imgs; offset, read_σ, gain);
end;
```

### 2.3 Task - Calculate mean and variance

Calculate the mean and variance of those images, but only along the third dimension! Either use a library (Statistics) or write it yourself:)

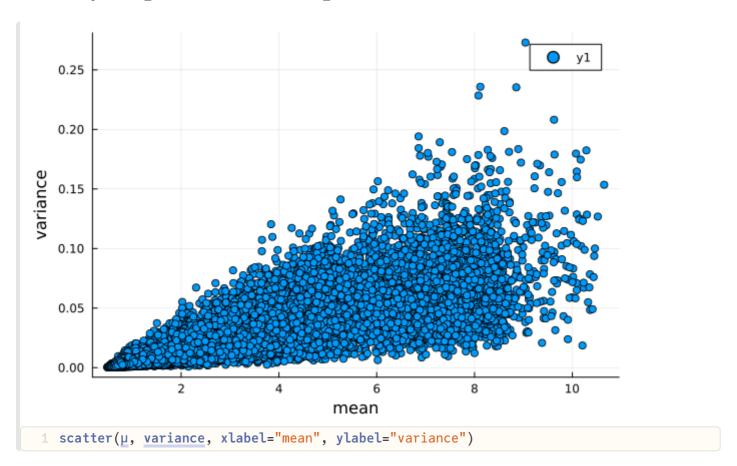
In this case, the mean along the third dimension would be

```
2×1×1 Array{Float64, 3}:
[:, :, 1] =
2.0
2.0
1 [2.0; 2.0;;;]
```

```
▶[0.79168, 0.793474, 0.908173, 0.882048, 1.02107, 1.05338, 1.13815, 1.29924, 1.37484, 1.5145

1 begin
2 # mean
3 µ_arr = mean(images, dims=3) # calculate the mean of 'images' along dim 3
4 µ = µ_arr[:]
5 end
```

### Finally, we produce a scatter plot



## 2.4 Fitting

Having our noisy dataset, we want to **extract the gain** of the detector since the gain translate from a photon count to an eletrical signal (ADU).

Why does plotting the **variance**  $\sigma^2$  over the **mean**  $\mu$  allows to fit the gain? We know, that in a Poisson distribution the expected mean  $\mu$  is always equal to the variance  $\sigma^2$ .

$$\sigma^2 = \mu$$

If we now take 10 captures of the same image, we always expect the same mean  $\mu$  for a certain pixel. Of course, those are 10 evaluation of a random measurement process, therefore we observe fluctuations. Hence, for each pixel we can calculate the mean and the variance.

Since the detector multiplies the measured photons with a certain gain, we do not measure the photons but the digital value. Hence the **measured mean** is

$$\hat{\mu} = \text{gain} \cdot \mu = \text{gain} \cdot \sigma^2$$

where  $\mu$  would be the **expected photon number**.

However, the measured variance is

$$\hat{\sigma}^2 = \sum_i (\hat{\mu} - \mathrm{gain} \cdot x_i)^2 = \mathrm{gain}^2 \sigma^2$$

where  $\sigma^2$  would be the variance of the photon number.

$$\hat{\sigma}^2 = \operatorname{gain} \cdot \hat{\mu} + \operatorname{offset}$$

The slope of our variance over mean is then

$$rac{\Delta\hat{\sigma}^2}{\Delta\hat{\mu}}=\mathrm{gain}$$

Dividing  $\hat{\mu}$  by **gain** returns the measured photon number.

```
1 md" ## 2.4 Fitting
```

2 Having our noisy dataset, we want to \*\*extract the 'gain'\*\* of the detector since the gain translate from a photon count to an eletrical signal (ADU).

4 Why does plotting the \*\*variance\*\* \$\sigma^2\$ over the \*\*mean\*\* \$\mu\$ allows to fit the gain?

5 We know, that in a Poisson distribution the expected mean \$\mu\$ is always equal to the variance \$\sigma^2\$.

 $7 \leq \infty = \mu$ 

9 If we now take 10 captures of the same image, we always expect the same mean \$\mu\$ for a certain pixel. Of course, those are 10 evaluation of a random measurement process, therefore we observe fluctuations. Hence, for each pixel we can calculate the mean and the variance.

```
Since the detector multiplies the measured photons with a certain gain, we do not
measure the photons but the digital value.
Hence the **measured mean** is

$\\hat \mu = \text{gain} \cdot \mu = \text{gain} \cdot \sigma^2$

where $\mu$ would be the **expected photon number**.

However, the **measured variance** is

$\\hat \sigma^2 = \sum_{i} (\hat \mu - \text{gain} \cdot x_i)^2 = \text{gain}^2
\sigma^2$\\
where $\sigma^2$\\
where $\sigma^2$\\
where $\sigma^2$\\
where $\sigma^2$\\
hat\sigma^2 = \text{gain} \cdot \hat\mu + \text{offset}\\
The slope of our variance over mean is then

$\\\frac{\text{facin}}{\text{cot}} \text{gain} \shat \mu \ \mu = \text{gain}\\
Dividing $\hat \mu$ by $\text{gain}$ returns the measured photon number.
```

```
1 # for fitting
2 using LsqFit
```

## 2.5 LsqFit.jl

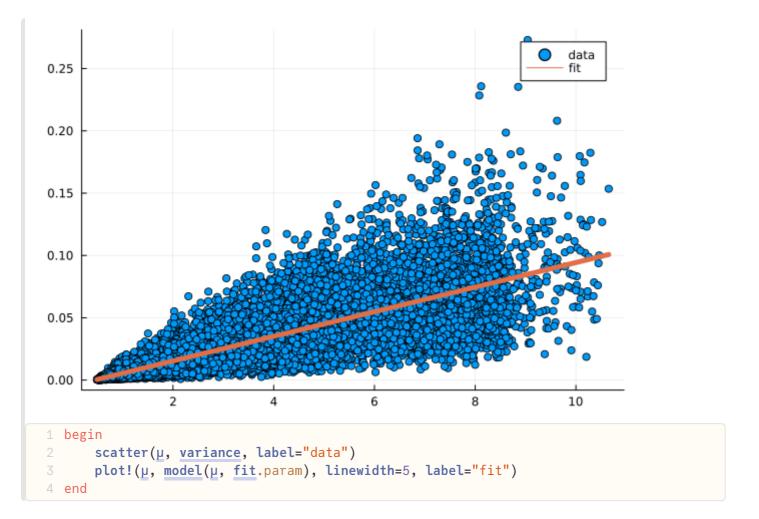
model (generic function with 1 method)

We now want to fit a linear function with a slope and a offset on our data. Try to find out <u>here</u> how that works

```
1 @. model(x, p) = p[1] .* x .+ p[2] #p[1] is gain, p[2] offset

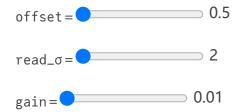
p0 = ▶[0.5, 0.5]

1 p0 = [0.5, 0.5] # TODO (maybe you need to convert it to Float32, maybe not), initial guess
```



### **Final Check**

If you slide those parameters, you change the simulated values.



The final solution is gain =  $0.009871 \pm 4.7e-5$ 

```
1 md"The final solution is gain = $(round(fit.param[1], sigdigits=4)) ±
$(round(stderror(fit)[1], sigdigits=2))"
```